

Table S1: Summary of genetic interactions between PS and APP, and PS and motor proteins.

	AppL ^{-/Y}	AppL ^{-/+} ;psnl ^{-/+}	psnl ^{-/-}	psnl ^{[143]^{-/-}}	psnl ^{-/+} ;syd ^{-/+}	Syd ^{-/-}	psnl ^{-/+} ;khc ^{-/+}	Khc ^{-/-}	psnl ^{-/+} ;kic ^{-/+}	Kic ^{-/+}	Kic ^{-/-}	psnl ^{-/+} ;roblik ^{-/+}	Roblik ^{-/+}	Roblik ^{-/-}	Wild Type	
Axonal defects	YES ↑	NO	-	NO	NO	NO ↑	NO	NO	NO	NO	NO ↑	NO	NO	NO	NO ↑	NO
Adult viable	YES	NO	YES	NO, Early lethal	NO	YES	YES	NO	YES	YES	NO, Early larval lethal	YES	YES	NO	YES	YES NO, larval lethal

Table S2: Summary of genetic interactions between hAPP or SWE, and PS and the other components of the gamma secretase complex. (ND= not determined)

	hAPP	SWE	hAPP;psnl ^{-/+}	SWE;psnl ^{-/+}	hAPP;aph-1 ^{-/+}	aph ^{-/+}	hAPP;nct ^{-/+}	nct ^{-/+}	hAPP;pen2 ^{-/+}	Pen2 ^{-/+}	hAPP;kinesin ^{-/+}	
Axonal defects	YES	YES	NO	NO	YES	NO	YES	NO	YES	NO	NO ↑	
Cell death	YES	YES	NO	NO	ND	ND	ND	ND	ND	ND	ND ↑	
Adult viable	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO, larval lethal	

Table S4: Summary of *in vivo* motility data

	Anterograde velocity	Retrograde velocity
APP-YFP	normal	normal
APP-YFP;psnl -/+	↑	↑
APP-YFP;g-secretase inhibitor	↑	↑
APP-YFP;kinesin-/+	↓	normal
APP-YFP;dynein-/+	↓	↓
SYNT-EGFP	normal	normal
SYNT-EGFP;psnl -/+	normal	normal

Table S5: Summary of mode analysis**1. APPYFP**

		MEAN	STD	Weight
Anterograde	Mode 1	0.3435 µ/sec	0.0806	18.47%
	Mode 2	0.6196 µ/sec	0.1749	45.93%
	Mode 3	1.3461 µ/sec	0.5430	35.60%
Retrograde	Mode 1	0.3832 µ/sec	0.1060	50.07%
	Mode 2	0.7827 µ/sec	0.2532	41.19%
	Mode 3	1.3373 µ/sec	0.4254	8.74%

2. APPYFP; *psnI*-/+

		MEAN	STD	Weight
Anterograde	Mode 1	0.5337 µ/sec	0.1652	45.61%
	Mode 2	1.1354 µ/sec	0.3754	37.35%
	Mode 3	2.5073 µ/sec	0.8080	17.04%
Retrograde	Mode 1	0.5171 µ/sec	0.1284	36.14%
	Mode 2	0.8410 µ/sec	0.2331	40.44%
	Mode 3	1.8476 µ/sec	0.6593	23.42%

3. *psnI*-/+; *roblk*-/+

		MEAN	STD	Weight
Anterograde	Mode 1	0.4384 µ/sec	0.1183	39.15%
	Mode 2	0.7212 µ/sec	0.2167	41.62%
	Mode 3	1.5707 µ/sec	0.6472	19.22%
Retrograde	Mode 1	0.4176 µ/sec	0.1167	29.09%
	Mode 2	0.7188 µ/sec	0.2085	49.77%
	Mode 3	1.5254 µ/sec	0.5515	21.13%

4. *psnI*-/+; *khc8*-/+

		MEAN	STD	Weight
Anterograde	Mode 1	0.3677 µ/sec	0.1066	29.85%
	Mode 2	0.6510 µ/sec	0.2174	51.95%
	Mode 3	1.4625 µ/sec	0.6298	18.20%
Retrograde	Mode 1	0.4840 µ/sec	0.1581	47.84%
	Mode 2	0.8515 µ/sec	0.2664	25.63%
	Mode 3	1.5020 µ/sec	0.5273	26.54%

Table S3: Summary of in vivo measurements from the custom single particle tracking software program.

	APPYFP	APPYFP; PSNI-/+	APPYFP; PSN[143]-/ +/-	SYNT- EGFP	SYNT- EGFP; PSNI-/+	APPYFP; PSNI-/+; KHC8-/+	APPYFP; PSNI-/+; ROBLK-/+	APPYFP; NCT-/+	APPYFP; PEN-/+	APPYFP; APH-/+	1uM compound E	10uM compound E	50uM compound E
Total number of vesicles	524	1231	338	179	246	862	638	165	508	341	375	213	368
Stationary vesicles	163	226	92	87	154	410	248	54	115	99	171	75	133
Anterograde vesicles	151	673	120	13	30	203	202	26	166	89	100	61	74
Retrograde vesicles	127	108	79	42	25	100	54	35	93	76	49	26	65
Switching vesicles	83	224	47	37	37	149	134	50	134	77	55	51	96
Anterograde duration-weighted segmental velocity (mean±s.d.; µm/sec)	1.03±0.55 N = 229 Tracks	1.42±0.90 N = 892	0.98±0.55 N = 166	0.68±0.36 N = 48	0.71±0.39 N = 65	0.87±0.57 N = 346	0.98±0.62 N = 334	0.83±0.47 N = 73	1.11±0.68 N = 292	1.10±0.58 N = 162	1.22±0.69 N = 151	1.30±0.89 N = 108	1.03±0.65 N = 165
Retrograde duration-weighted segmental velocity (mean±s.d.; µm/sec)	0.76±0.38 N = 204 tracks	1.13±0.68 N = 302	0.95±0.45 N = 124	0.99±0.43 N = 79	0.88±0.37 N = 61	1.02±0.55 N = 244	0.92±0.52 N = 181	0.87±0.44 N = 84	0.94±0.49 N = 217	1.17±0.57 N = 146	1.33±0.68 N = 101	1.07±0.59 N = 77	0.96±0.51 N = 154
Anterograde unweighted segmental velocity (mean±s.d.; µm/sec)	0.83±0.53 N = 457 segments	1.09±0.81 N = 1597	0.77±0.50 N = 374	0.62±0.42 N = 110	0.63±0.40 N = 155	0.71±0.49 N = 799	0.77±0.52 N = 857	0.70±0.42 N = 178	0.89±0.63 N = 586	0.90±0.57 N = 322	0.92±0.62 N = 316	1.00±0.77 N = 219	0.84±0.56 N = 360
Retrograde unweighted segmental velocity (mean±s.d.; µm/sec)	0.63±0.36 N = 512 segments	0.96±0.63 N = 528	0.82±0.46 N = 264	0.86±0.45 N = 176	0.80±0.48 N = 135	0.85±0.53 N = 499	0.80±0.50 N = 427	0.75±0.46 N = 184	0.80±0.47 N = 453	0.97±0.54 N = 276	1.15±0.69 N = 179	0.92±0.56 N = 157	0.83±0.50 N = 303
Anterograde segmental pause frequency (mean±s.e.m.; sec ⁻¹)	1.93±0.18 N = 275 Pauses	1.49±0.09 N = 739	1.75±0.18 N = 246	2.34±0.37 N = 75	2.16±0.29 N = 108	1.86±0.12 N = 536	1.55±0.10 N = 526	2.07±0.30 N = 102	1.57±0.14 N = 313	1.69±0.20 N = 145	1.89±0.21 N = 181	1.54±0.24 N = 105	1.78±0.19 N = 175
Retrograde segmental pause frequency (mean±s.e.m.; sec ⁻¹)	1.67±0.14 N = 353 pauses	1.72±0.19 N = 196	1.42±0.20 N = 143	1.46±0.24 N = 102	2.18±0.34 N = 83	1.80±0.17 N = 251	1.77±0.16 N = 230	1.39±0.22 N = 88	1.76±0.18 N = 244	1.33±0.21 N = 100	1.82±0.32 N = 69	1.64±0.31 N = 64	1.78±0.22 N = 138
Anterograde pause	1.49±0.07	1.28±0.03	1.51±0.07	1.87±0.21	1.99±0.19	1.95±0.08	1.40±0.05	1.60±0.12	1.44±0.06	1.51±0.10	1.38±0.09	1.33±0.10	1.56±0.12

duration (mean±s.e. m.; sec)	N = 275 pauses	N = 739	N = 246	N = 75	N = 108	N = 536	N = 526	N = 102	N = 313	N = 145	N = 181	N = 105	N = 175
Retrograde pause duration (mean±s.e. m.; sec)	1.52±0.07 N = 353 pauses	1.20±0.06 N = 196	1.52±0.10 N = 143	1.46±0.10 N = 102	1.73±0.17 N = 83	1.42±0.06 N = 251	1.40±0.06 N = 230	1.54±0.10 N = 88	1.29±0.06 N = 244	1.40±0.11 N = 100	1.19±0.08 N = 69	1.23±0.08 N = 64	1.64±0.15 N = 138
Anterograde velocity mode 1 (velocity and percentage of vesicles in mode)*	0.34±0.08 18.47%	0.54±0.17 45.61%	0.32±0.02 5.30%	0.36±0.12 37.27%	0.39±.012 41.46%	0.37±0.11 29.85%	0.44±0.12 39.15%	0.39±0.12 43.72%	0.48±0.16 50.24%	0.43±0.14 33.09%	0.34±0.05 8.73%	0.58±0.19 63.02%	0.51±0.16 51.54%
Anterograde velocity mode 2 (velocity and percentage of vesicles in mode)*	0.62±0.17 45.93%	1.14±0.38 37.35%	0.53±0.17 63.83%	0.65±0.19 52.49%	0.71±0.25 53.13%	0.65±0.22 51.95%	0.72±0.22 41.62%	0.81±0.23 46.87%	1.03±0.37 36.27%	0.87±0.28 46.82%	0.60±0.18 57.87%	1.05±0.34 19.75%	0.85±0.25 31.69%
Anterograde velocity mode 3 (velocity and percentage of vesicles in mode)*	1.35±0.54 35.60%	2.51±0.81 17.04%	1.34±0.53 30.87%	1.46±0.69 10.24%	1.75±0.71 5.41%	1.46±0.63 18.20%	1.57±0.65 19.22%	1.57±0.54 9.41%	2.03±0.70 13.48%	1.72±0.59 20.09%	1.62±0.60 33.39%	2.50±0.51 17.22%	1.81±0.63 16.77%
Retrograde velocity three-mode fitting: mode 1 (velocity and percentage of vesicles in mode)*	0.38±0.11 50.07%	0.52±0.13 36.14%	0.48±0.15 47.57%	0.51±0.17 40.17%	0.43±0.12 35.42%	0.48±0.16 47.84%	0.42±0.12 29.09%	0.38±0.15 29.04%	0.38±0.09 24.68%	0.56±0.19 37.35%	0.56±0.14 41.17%	0.42±0.14 32.84%	0.53±0.18 45.77%
Retrograde velocity three-mode fitting: mode 2 (velocity and percentage of vesicles in mode)*	0.78±0.25 41.19%	0.84±0.23 40.44%	1.10±0.38 51.67%	1.03±0.34 56.93%	0.85±0.23 50.94%	0.85±0.27 25.63%	0.72±0.21 49.77%	0.71±0.23 46.92%	0.66±0.17 44.25%	1.11±0.38 55.88%	1.08±0.29 21.97%	0.78±0.14 33.03%	0.84±0.27 33.44%

Retrograde velocity three-mode fitting: mode 3 (velocity and percentage of vesicles in mode)*	1.34 ± 0.43 8.74%	1.85 ± 0.66 23.42%	2.73 ± 0.09 0.75%	2.25 ± 0.35 2.89%	1.57 ± 0.67 13.64%	1.50 ± 0.53 26.54%	1.53 ± 0.55 21.13%	1.30 ± 0.52 24.03%	1.32 ± 0.46 31.07%	2.01 ± 0.88 6.77%	1.84 ± 0.59 36.86%	1.54 ± 0.50 34.14%	1.51 ± 0.59 20.78%
-----------------------------------------------------------------------------------------------	--------------------------	---------------------------	--------------------------	--------------------------	---------------------------	---------------------------	---------------------------	---------------------------	---------------------------	--------------------------	---------------------------	---------------------------	---------------------------

*: All velocity modes are in $\mu\text{m/sec}$.